

The Reproductive Biology of *Durio zibethinus* Murr.

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SUMMARY

Durio zibethinus Murr. or the common durian is a fruit-tree species widely cultivated in villages or orchards or a semi-wild plant found growing around aborigines' settlements in Peninsular Malaysia. The species is generally considered by botanists as a native tree in Borneo and Sumatra, though currently it is commonly planted throughout South East Asia, extending from the south-eastern parts of India to New Guinea.

In Peninsular Malaysia the flowering is seasonal and normally falls during the months of March-April and September-October, though accessory flowering may take place in between. Development of flowers takes about five to seven weeks and the flowering lasts for about three weeks. Floral parts develop acropetally and the epicalyx, calyx, corolla and stamens fall off soon after anthesis. Floral anthesis is initiated at about 16.00 hrs. and completed by about 20.00 hrs. Pollination is carried out by nectarivorous bats (*Eonycteris spelaea*) and by an unidentified noctuid moth, and takes place between 20.00 and 01.00 hrs. Pollen grains are more or less spherical, 80-150 μ in diameter, 3-4- or rarely 6-porate, with a smooth but sticky exine; kept under room temperature they remain viable for about 48 hrs. The flower is self-compatible, though the percentage of successful fertilisation and production of fruit reaching maturity increase if the flowers are crossed.

The anthers though initially tetrasporangiate become bisporangiate at maturity. Wall development conforms with the basic type. Before anthesis the epidermis is made up of more or less rectangular and isodiametric cells, but towards anthesis these cells become papillate and filled with tannin, and eventually shed off from the anther wall. The endothelial cells become fibrous and both the middle layers and the tapetal layer are crushed and disappear, leaving the endothecium the only wall enclosing the pollen grains. Cytokinesis of microspore mother cells is simultaneous and gives rise to tetrahedral tetrads. Anther dehiscence is through a longitudinal slit caused by the breakdown of the wall at the meeting point of the anther-lobes. Pollen grains are binucleate at the time of shedding.

The ovule is anatropous, bitegmic and crassinucellate. The micropyle is formed by both integuments. Embryo-sac development conforms with the *Polygonum*-type. Antipodal cells are ephemeral.

The seed is arillate and its mode of germination is epigeal and takes place within three days after sowing in garden soil. Seed viability can be prolonged up to 32 days (90% germination) if the seeds are surface-sterilised, kept in an air-tight container and placed under 20°C.

INTRODUCTION

Previous works on *Durio* (Wyatt-Smith, 1953; Kostermans, 1958; Reksodi-hardjo, 1962 and Kochummen, 1972) indicate that there are at least 28 species in the genus, distributed throughout Burma, Thailand, Peninsular Malaysia, Singapore, Sumatra, Borneo and Palawan Island. Though considered by botanists as a tree native to Sumatra and Borneo, *Durio zibethinus* or the common durian is now widely cultivated as a fruit-tree in the South Asia region, covering the south-eastern parts of India, Ceylon, Burma, Thailand, Indo-china, Malaysia, Singapore, Indonesia, the Philippines and New Guinea.

Species of *Durio* are found growing naturally in lowland and hill primary forests (up to 1000 m altitude) usually not more than 3 to 4 trees per hectare. Apart from *Durio zibethinus* there are five other species which produce edible

fruits (Reksodihardjo, 1962). These are *D. dulcis* Becc. (found in Sabah and Indonesian Borneo), *D. grandiflorus* (Mast.) Kost. (Sabah, Sarawak and the Indonesian Borneo), *D. graveolens* Becc. (Peninsular Malaysia, throughout Borneo and Sumatra), *D. kutejensis* (Hassk.) Becc. (throughout Borneo), and *D. oxleyanus* Griff. (Peninsular Malaysia, throughout Borneo and Sumatra). All five are cultivated in Brunei and some to a limited extent elsewhere in Malaysian Borneo. The other species of the genus, though not producing edible fruits, possess several desirable features for breeding and bud-grafting purposes. These features are: disease and pest resistance (most wild species), more regular flowering (*D. acutifolius* (Mast.) Kost. & *D. griffithii* (Mast.) Bakh.), flowers and fruits borne on the lower parts of the stem (*D. beccarianus* Kost. & Soeg., *pinangianus* (Becc.) Ridl. and *testudinarum* Becc.), or on the stem as well as on the lower branches (*D. malaccensis* Mast.). Though this does not necessarily mean that all species are easily hybridised, it does imply that, since the specific delimitation of the genus is mainly based on morphological attributes and since there are several species which are closely related to each other (e.g. *D. zibethinus*, *malaccensis* and *wyatt-smithii* Kost.), and, there seem to be many intermediate forms among natural populations of and between species, there is a possibility to improve the quality as well as the productivity of the existing edible-fruit producing species, at least by bud-grafting.

In spite of the fact that durian fruit is of high economic importance to local inhabitants (Lai, 1974), as far as we know there is no large scale plantation or estate in the region, nor is there a well documented and systematic breeding and selection programme. This lack of interest may partly be due to the fact that very little is known about the autecology, flowering biology, cytology and breeding system of the species. The only paper dealing with some aspects of reproductive biology of *Durio* species so far published is that by Valvayor, Coronel & Ramirez in 1965.

It is therefore the aim of the present study to gather more information about *D. zibethinus* and its related species so that their economic potential and contribution to "durianology" in general is not completely forgotten.

MATERIALS & METHODS

Field work to determine the distribution and frequency and to observe the phenology, floral anthesis and pollination processes of *D. zibethinus* and its related species was carried out in the University of Malaya campus, Damansara village, Ulu Gombak, Mantin and Kuala Selangor (all in Selangor State), Kuala Pilah and Pasoh Forest Reserve (in Negri Sembilan), Krau Game Reserve, Taman Negara and Tioman Island (Pahang). For detailed studies on the floral anthesis and for pollination experiments, a tree growing in the compound of the Faculty of Agriculture, University of Malaya was used.

Flowers of different developmental stages were collected regularly during the flowering periods, fixed in 50% F.A.A. solution and then sectioned and stained according to normal schedules. Guano samples were collected weekly from Cavern C of the Batu Caves Limestone Hill from February 1974 to January 1975. Pollen content was extracted from these samples and acetolysed and then stained with safranin.

OBSERVATIONS & RESULTS

Phenology. Depending on the clones, soil and climatic condition in which the durian tree is planted, it starts to bear flowers and fruits at the age of 5 to 12 years. In Peninsular Malaysia there seem to be two main flowering seasons, normally falling in the period of March-April and September-October. However, it should be noted that minor or accessory flowering may occur in between. The flowers are born in fascicles of 3-30 on the older branches. Flowers of the same

inflorescence usually mature more or less at the same time and open one after another within a few days. Since during the flowering each individual tree produces hundreds of flowers, and the maturation of the flowers of different inflorescences is not necessarily synchronised, the flowering period of a particular season usually lasts for about two or three weeks. It was also observed that normally the first flowering of a particular year is heavier than the second. What causes this remains to be investigated. The fruit set is usually very low since many of the ovaries will drop after anthesis, either because their ovules are not fertilised or have been disturbed or destroyed by the pollinators. Fruits take approximately three months to reach maturity.

Floral morphology and development. At its early stage of development, each individual flower-bud is a globose structure made up of a mass of homogeneous cells surrounded and enclosed by bracts and epicalyx. The sepaline, petaline, staminal and carpellary primordia develop acropetally at more or less the same rate. The anthers develop from the distal cells of the phalanges as globular protuberances. Each protuberance is composed of a homogeneous mass of meristematic cells surrounded by an epidermis. As the phalanges elongate and differentiate into distinct filaments the developing young anthers assume their 4-lobed appearance. Just before anthesis the buds attain a size of about 2 cm in diameter. Both the epicalyx and calyx are externally densely covered with brownish peltate fimbriate scales, and the petals are yellowish-white and sparsely hairy outside. The scales are multicellular and originate from the epidermal cells. The nectary is located at the inner basal part of the calyx-cup. At anthesis the flower reaches about 5-6 cm long and 2-3 cm in diameter and emits a strong odour reminiscent of sour milk but somewhat fragrant. The carpels develop and originate from a common primordium situated at the centre of the flower-bud. This primordium is made up of homogeneous and more or less isodiametric cells. These cells divide and differentiate into five carpels which fuse at their marginal and central parts to form a 5-loculate ovary with a central placental column. The style is formed by vertical growth of the five carpels and is topped by a capitate stigma. The stigmatic surface is uneven in outline with deep depressions or notches here and there (Plate 4a). By the time the megaspore mother cell is formed, the spine-primordia of the ovary wall start to develop. These primordia originate from the hypodermal layer and appear as conical protuberances each of which is topped by a multicellular, peltate and fimbriate scale similar to those of epicalyx and calyx. As the flower develops fully, cells in the tissues of the epicalyx, calyx and petals contain tannin and become mucilaginous.

Development of anther-wall. In each of the anther lobes and just below the epidermis, a row of hypodermal cells increase in size and contain more conspicuous nuclei and denser cytoplasm. These cells form the archesporial tissue. Each archesporial cell divides periclinally into a primary parietal cell and a primary sporogenous cell (Pl. 1a). The primary parietal cell further divides periclinally into two secondary parietal cells. The outer secondary parietal cell divides once again to give rise to an endothecial cell and outer middle-layer cell. The inner secondary parietal cell also divides further and produces the inner middle-layer cell and the tapetal cell. Thus the anther-wall formation conforms well with the basic type. By the time the sporogenous cell divides and produces numerous microspore mother cells, the anther wall is greatly stretched and the middle layers as well as the tapetum are crushed and their cells become flattened. Meanwhile through the disintegration of the septa separating the four original anther cavities, the anther becomes two-loculate. Towards the end of meiosis the epidermal cells become papillate and filled with tannin, and just before the anther dehisces they are shed off leaving the endothecial cells as the only surviving wall enclosing the pollen grains. At this stage the wall of the endothecial cells becomes fibrous and the wall thickening appears as radially oriented bar-like structures.

Microsporogenesis. By the time the anther wall attains its 4-cells thickness, the primary sporogenous cell divides into two daughter cells (Plate 1b). These cells divide both periclinally and anticlinally to form numerous microspore mother cells (Plate 1c & d). Meiotic division starts from those microspore mother cells situated at the centre of the anther cavity and progresses outwards (Plate 1e & f). Many of the peripheral microspore mother cells fail to complete the division and become abortive and assume a flat outline. The first division of the microspore mother cell is not immediately followed by wall formation (Plate 1e). The resulting four microspores are formed simultaneously and clustered in a tetrahedral arrangement (Plate 1f). At the time of shedding most of the pollen grains are binucleate. It may be noted that development as well as formation of microspores are not synchronised in all anthers of the same flower.

Pollen morphology. Mature pollen grains are more or less spherical, 3-4 rarely 6-porate, and measuring 80-150 μ in diameter (Plate 2a). The exine is very much thicker than the intine, smooth but covered with sticky substances, and thicker around the pores. At anthesis they are released singly or in clumps (Plate 2b).

Pollen germination. Pollen grains collected from the anthers at the beginning of floral anthesis do not show any sign of germination, but those collected from the fallen phalanges on the following morning start to germinate. Two hundred of these pollen grains were kept under room temperature, and after 40 hours from 23.5 to 80% of the pollen grains germinated. This seems to indicate that stigmatic exudate is not the sole prerequisite of germination and that kept under room temperature the pollen remain viable for at least 48 hours. Germination experiment using sucrose solution of various concentrations shows that after culturing the pollen for 12 hours, the optimal percentage of germination (c. 77%) takes place in 6% solution. In this experiment it was also observed that the higher the concentration of the sucrose, the longer the pollen-tube is.

Development of ovule. In each of the carpellary cavities two alternate rows of 5-7 ovular primordia appear from the central placental column as minute and somewhat conical protuberances (Plate 2c). Each primordium is at first composed of homogeneous, thin-walled and more or less isodiametric cells, but later one of the hypodermal cells becomes larger in size than the surrounding cells and contains dense cytoplasm and a more conspicuous nucleus (Plate 2c). This cell develops into the archesporial cell and divides periclinally into a primary parietal cell and sporogenous cell (Plate 2c). The primary parietal cell divides periclinally and anticlinally to form the 5-6 cells thick nucellus. Soon after the division of archesporial cells is completed, the integument primordia develop more or less simultaneously on both sides of the nucellus (Plate 2d). However, the outer integument grows faster and eventually overtops the inner one. The micropyle is formed by both the inner and outer integuments. At the formation of the megaspore mother cell the integuments are 2-3 cells thick, and later more cells are laid down. Several cells of the outer integument are eventually filled with tannin. It may be noted here that on two occasions binucellate ovules were observed. The two nucelli are enclosed by a common outer integument but each has its own inner integument.

Megasporogenesis. The sporogenous cell enlarges and functions as the megaspore mother cell (Plate 2d). This cell divides into two (not seen) and eventually into four daughter cells arranged in a linear tetrad (Plate 2e). Three of these daughter cells degenerate, leaving the cell at the chalazal end to develop further. This functional megaspore undergoes vacuolation and forms an elongated uninucleate embryo-sac. Subsequently it passes through two-, four- and eight-nucleate stages before cytokinesis commences (Plate 3a, b & c). One of the four micropylar daughter nuclei moves towards the centre of the embryo-sac, and the other three form the egg apparatus and two synergids. Similarly, one of the chalazal nuclei also moves to the centre of the embryo-sac while the other three form the ephemeral antipodals (Plate 3c & d). The two polar nuclei then fuse

with one another to form the secondary polar nucleus (Plates 4c & 5a). The development of the eight-nucleate embryo-sac, therefore, conforms well with the *Polygonum*-type.

Pollination. Opening of the flower usually takes place according to the following sequence: epicalyx splits into 2–3 ovate-concave lobes about 12–24 hours before anthesis; the calyx then splits open at its tip into 5–6 acute lobes about 8–10 hours before anthesis; for the next two hours or so the petals, styles and stamens which initially take an incurved position within the calyx become fully exerted and soon after dark the petal-lobes become recurved outwards exposing both stamens and styles; meanwhile some of the anthers may start to dehisce but the majority do not do so before c. 19.30 hrs; the stigmatic surface becomes receptive at about 20.00 hrs. The flower remains at this stage until about 01.00 hrs., and then the calyx, petals and stamens begin to drop off, leaving the lone ovary remains attached to the branch. Though initially many of these ovaries remain attached to the branch following pollination, within a few days most of them drop off and leave only 1–2 per inflorescence.

During the late afternoon, the flowers are visited by various insects as they open. Among these are honey bees, house-flies, lady-bird beetles, scarab beetles, and lacewings. Pollen grains were found on the legs and bodies of these insects but not in their guts. Since these insects visit the flowers before the latter reach full anthesis, they cannot be considered as pollinators. In the evening, namely between 20.00 and 01.00 hrs, the flowers are visited by three different species of bats. These are the nectarivorous bat (*Eonycteris spelaea*) and the frugivorous bats *Cynopterus brachyotis* and *Pteropus vampyrus*. Occasionally nocturnal moths also visit the flowers during this period. Among the bats, only *Eonycteris spelaea* could be considered as the genuine pollinator, since the other two directly feed on and chew up the flowers (Start, 1974). Our observation suggests that *Eonycteris spelaea* feeds on nectar as well as on pollen grains, and it does not chew the flower. The bats also visit the flowers regularly during the flowering season; they land on and clutch the flowers with the frontal parts of their body facing the open flowers. Analysis of guano samples also confirms that pollen grain of *Durio zibethinus* constitutes a significant part of the bat's diet during durian flowering season and that the highest number of grains in the samples coincides well with the flowering season of the trees. Other important pollen grains found constantly in the guano samples are those of *Parkia*, *Ceiba pentandra* (L.) Gaertn., *Bombax vuletonii* Hochr., *Oroxylon indicum* Vent., *Duabanga grandiflora* (Roxb. ex DC.) Walp., *Artocarpus* spp. (Plate 6a-d). This suggests that the bats feed on nectar or pollen, or both, of different species of plants which flower at different times of the year, and pollinate the flowers.

Pollination experiments. To test the compatibility of the flowers, a series of preliminary experiments were carried out during the flowering seasons in 1974. In each experiment a set of 20 flowers having a similar stage of development were selected and tagged. These flowers were then given the following treatments: (1) all anthers were removed at anthesis and the stigmas were exposed to natural pollinators, (2) the flowers were bagged before anthesis, (3) the stigmas were applied with Cutex nail varnish to prevent pollination, (4) the flowers were self-pollinated by hand and bagged, and (5) the anthers were removed and the stigmas were cross-pollinated by hand with pollen of other flowers of the same tree, and then bagged. At the beginning of these experiments the ovaries of all flowers used were between 0.35 and 0.4 cm in diameter and light-brown in colour. After 5 days all tagged flowers were re-examined and the following results were obtained: in treatment no. 1, 45% of the ovaries remained attached to the branch and showed further development, i.e. increase in diameter (0.5–0.6 cm) and change in colour to olive green; in experiment no. 2, only 15% of the ovaries showed further sign of development and the others either shrivelled or fell off; in the 3rd treatment

none of the ovaries underwent further development and shrivelled or dropped off; in the 4th, 50% of the ovaries exhibited further development and remained attached to the branch; and in the 5th 65% of the ovaries underwent further development and remained attached to the branch. At the end of the flowering season only 5% of the successfully pollinated ovaries developed into mature fruits.

The above experiments seem to suggest that (i) natural pollinators contribute at least 45% of the successful pollination, (ii) natural self-pollination can take place and contribute to 15% successful pollination, (iii) pollination is a prerequisite of fruit development, (iv) up to 50% of the flowers are self-compatible, and (v) cross-pollination between flowers of the same tree is the better means for successful fertilisation and eventual fruit development.

However, it should be emphasised here that since the number of flowers used in the experiments is small and the work was conducted on a single tree only, the above mentioned results should be considered as tentative. Future experiments using larger number of flowers and trees will either confirm or contradict the above results.

Fertilisation. The receptive stigma is heavily papillate and has a glistening and sticky surface. Pollen grains deposited on the stigmatic surface germinate within 3 or 4 hours. The germinating pollen grains are mostly monosiphonous, and the tubes make their way through the stigmatic papillae into the style. The pollen tubes grow downwards through the intercellular spaces of the vertically elongated protoplasmic cells of the transmitting tissue (Plate 4b). The successful tube enters the embryo-sac through the micropyle (Plate 4d). Although hundreds of slides were examined by us, the actual process of fertilisation has not been observed in detail. From the specimens available it seems that just before fertilisation the secondary polar nucleus moves nearer to the egg apparatus (Plates 4e & 5a).

Endosperm. The secondary polar nucleus which is situated near to the egg apparatus is then fertilised by one of the male gametes to form the primary endosperm cell (Plate 5a). This cell enlarges and undergoes free nuclear division. Most of the nuclei produced are distributed along the periphery of the embryo-sac and aggregated mainly at the chalazal end (Plate 5b). The endosperm remains in a free nuclear condition until a late stage of embryogeny and then becomes cellular.

Development of embryo and seed. In the present study the embryogeny has not been followed in detail. Sections of developing seed indicate that the endosperm does not persist and the cotyledons occupy the greater part of the seed cavity. The starchy food reserve is therefore stored in the cotyledons. Cells of the inner integument are crushed and disappear, and those of the outer integument become fibrous with the epidermal cells developing into rectangular and heavily lignified stone cells each with a very small lumen. The aril develops from the funicular end and eventually completely encloses the seed. This aril is very variable in thickness, colour, taste, smell and moisture content. It may be noted here that in a few clones, there is a high incidence of seed abortion, in which the seeds shrivel and measure less than 4 by 1.5 cm, while fully developed and viable seeds measure up to 7 by 4 cm.

Seed germination. The first sign of germination is indicated by cracking of the hilum at the micropylar end, and this takes place within 3 to 4 days after sowing the seeds in suitable medium. The radicle will emerge from this crack, elongate and grow downwards. After approximately 10 days numerous lateral roots appear at the proximal end of the radicle and the hypocotyl elongates and straightens up bringing the cotyledons still enclosed by the testa slightly above the soil surface. Subsequently the petioles or stalks of the cotyledons elongate allowing the plumule to emerge. The plumule elongates and from it the first and second leaves appear. These leaves are much smaller than the normal leaves and are deciduous. The cotyledons shrivel and drop off within 38 days following germination.

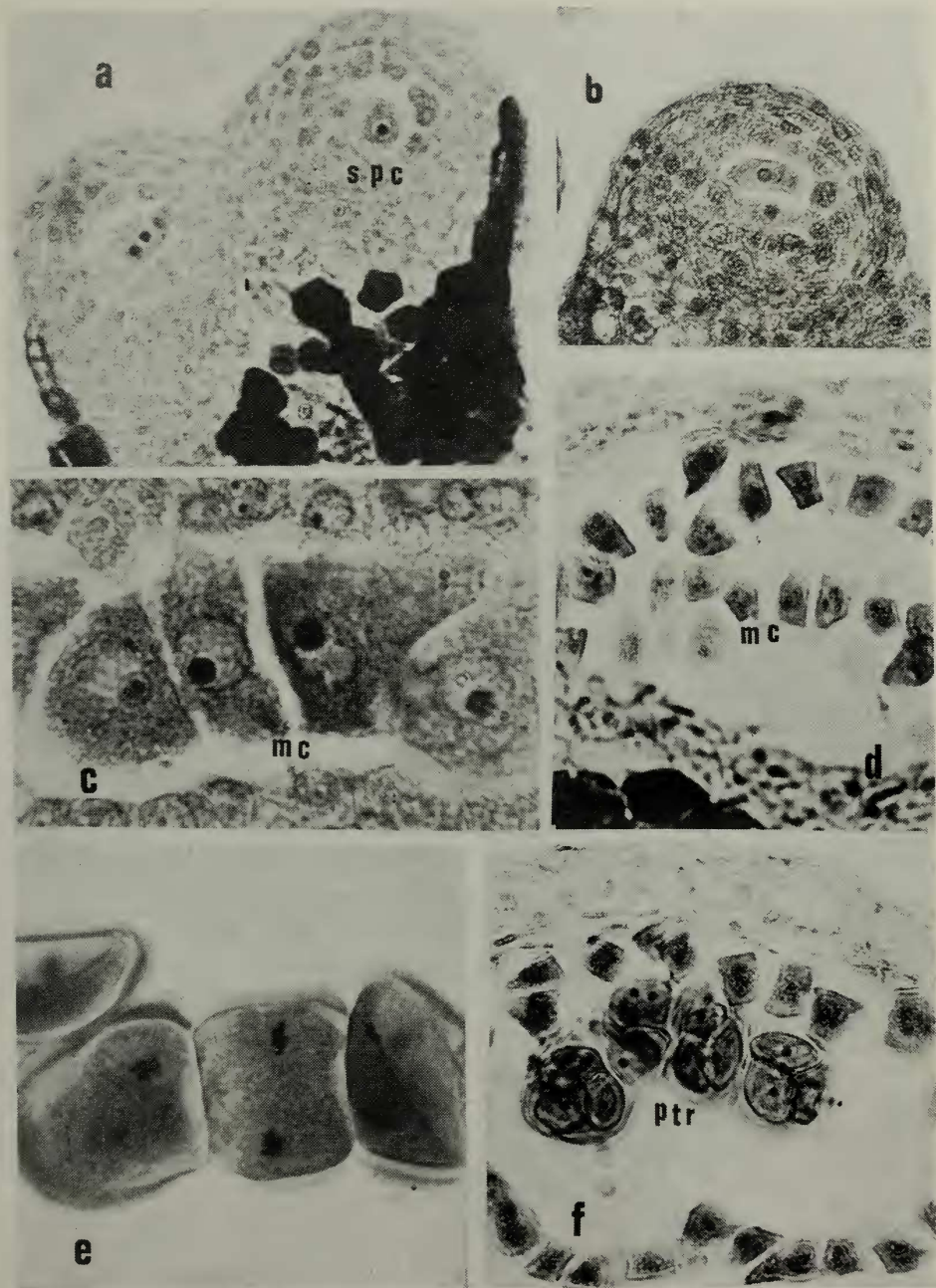


Plate 1: *a* & *b* developing anthers with sporogenous cell (s.p.c); *c* & *d* dividing and developing microspore mother cells (m.c); *e* division of microspore mother cells; *f* end of meiosis and formation of pollen tetrads (ptr).

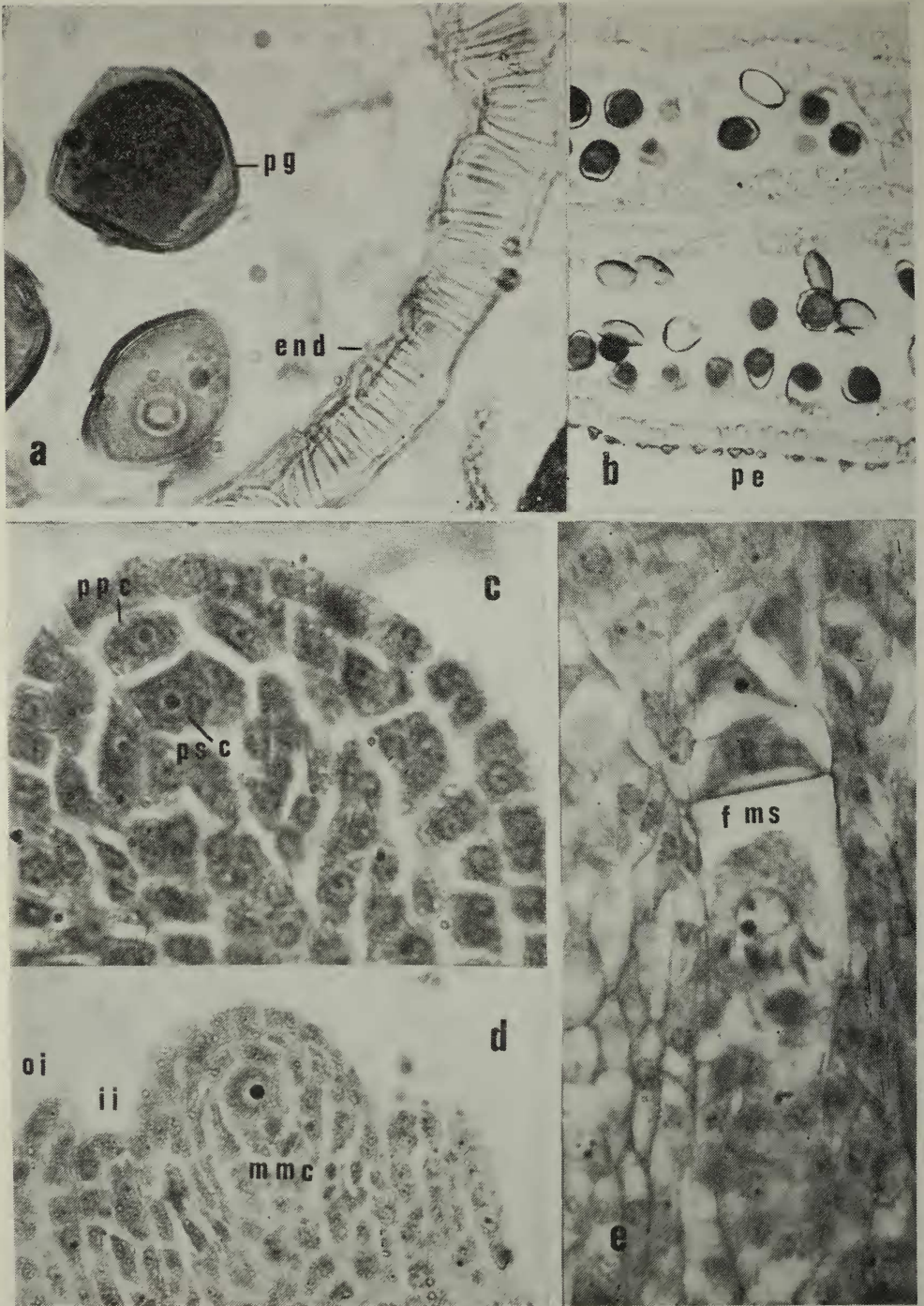


Plate 2: *a* & *b* cross-sections of anthers just before anthesis showing fibrous endothecium and mature pollen grains; *c* ovule primordium showing primary parietal cell (ppc) and primary sporogenous cell (psc); *d* ovule primordium showing developing megaspore mother cell (mmc) and integuments; *e* linear tetrad and functional megaspore (fms).

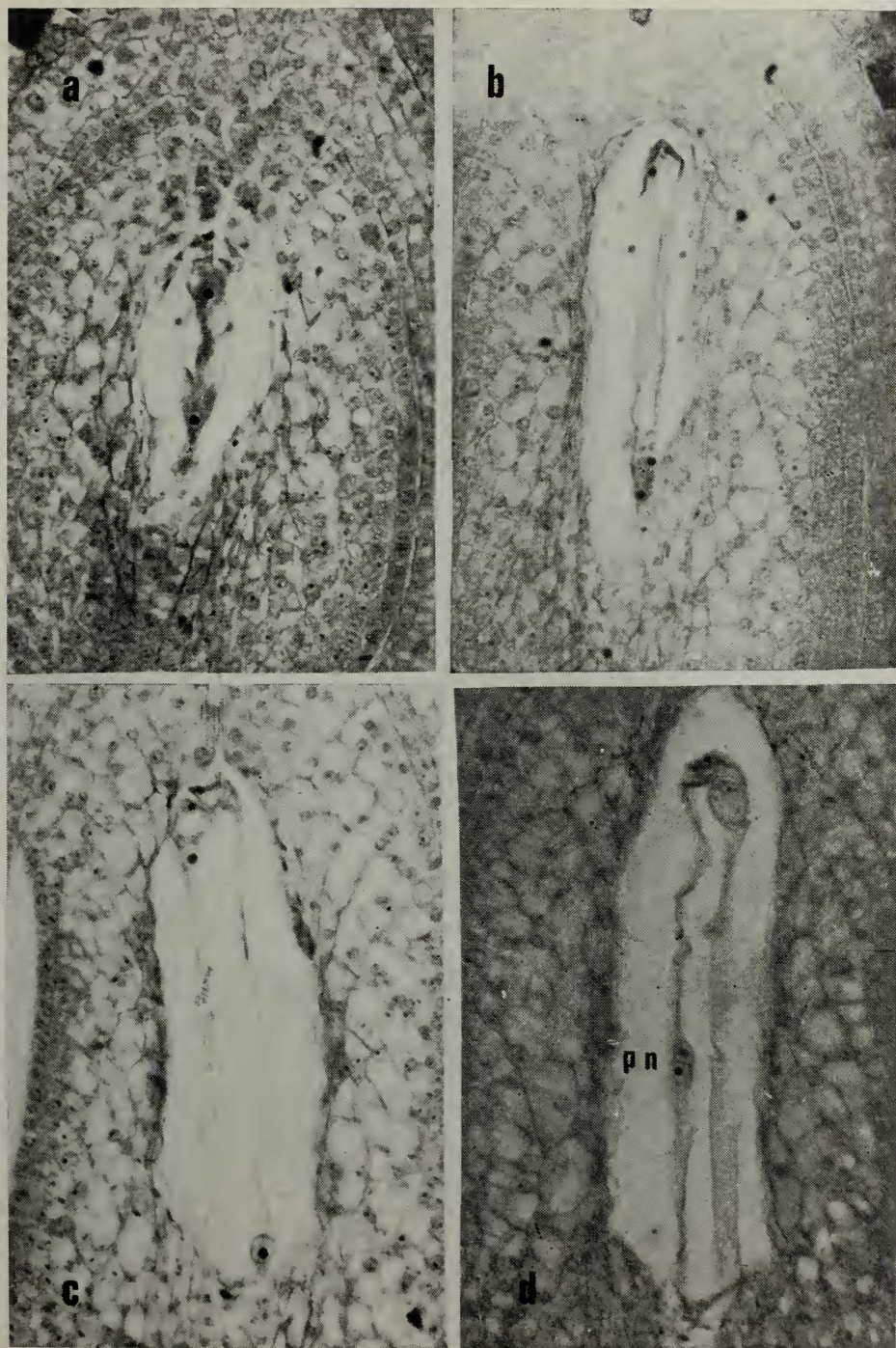


Plate 3: *a* 2-nucleate embryo-sac; *b* 4-nucleate embryo-sac; *c* & *d* 8-nucleate embryo-sac;
pn = polar nuclei.